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Short Communication

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A quantitative evaluation of the histological type dependence of the programmed death-ligand 1 expression in non-small cell lung cancer including various adenocarcinoma subtypes: a cross-sectional study

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Abstract

The association between non-small cell lung cancer histology and programmed death-ligand 1 expression remains controversial. We retrospectively analyzed histological dependence of the programmed death-ligand 1 expression by a multiple regression analysis of 356 non-small cell lung cancer patients. The programmed death-ligand 1 expression patterns of adenocarcinoma were consistent with a pathological predominant growth pattern as a reference to papillary adenocarcinoma: minimally invasive adenocarcinoma[partial regression coefficient (B), 0.17; 95% confidence interval, 0.05–0.59], lepidic adenocarcinoma (B, 0.46; 95% confidence interval, 0.23–0.90), acinar adenocarcinoma (B, 1.98; 95% confidence interval, 1.05–3.76) and solid adenocarcinoma (B, 5.11; 95% confidence interval, 2.20–11.9). In histology other than adenocarcinoma, the programmed death-ligand 1 expression tended to be high with poor differentiation: adenosquamous carcinoma (B, 4.17; 95% confidence interval, 1.05–16.6), squamous cell carcinoma (B, 4.32; 95% confidence interval, 2.45–7.62) and pleomorphic carcinoma (B, 13.0; 95% confidence interval, 4.43–38.2). We showed quantitatively that the programmed death-ligand 1 expression in non-small cell lung cancer tended to be clearly histology-dependent, with more poorly differentiated histology showing a higher expression.

Key words: programmed death-ligand 1, non-small cell lung cancer, adenocarcinoma subtypes, multiple regression analysis

Introduction

Lung cancer is the leading cause of cancer-related death worldwide, with >85% of cases classified as non-small cell lung cancer [NSCLC; (1)]. In recent years, immune checkpoint inhibitors have attracted much attention in the treatment of lung cancer. In particular, the expression of programmed death-ligand 1 (PD-L1) in tumors has been shown to be a clinical predictor of the therapeutic effect of immune checkpoint inhibitors on lung cancer (2,3). Therefore, the expression of PD-L1 is important to consider when predicting the treatment efficacy of immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway for NSCLC. However, there is limited information on the impact of multiple histological types and the degree of differentiation of NSCLC containing adenocarcinoma (ADC) subtypes, which are the most prevalent and abundant types of NSCLC, on the PD-L1 expression. We believe that this information may aid in screening patients for immune checkpoint inhibitor efficacy. This study statistically evaluated and clarified the effect of histology of NSCLC on PD-L1 expression in tumors, using surgically resected lung cancer specimens.

Materials and methods

Patients and pathological evaluations

The present study was approved by the Institutional Review Board of Kinki-Chuo Chest Medical Center (approval number: 738). Informed consent was obtained in the form of opt-out on the website of our institution. We included 356 NSCLC patients who had undergone surgical lung resection from 1 April 2016 to 31 January 2020 at our institution and had been examined for the PD-L1 expression in the resected tissue. The patients' age, gender and smoking status were collected from the medical records. The histology, PD-L1 expression and epidermal growth factor receptor gene (*EGFR*) mutations of the resected tissue were collected from the pathological report.

The *EGFR* mutation assay and tumor PD-L1 immunohistochemistry

All patients were subjected to an *EGFR* mutation assay by the testing laboratories (Cobas EGFR Mutation Test; Roche Molecular Diagnostics, Pleasanton, CA, USA) and PD-L1 immunohistochemistry 22C3 pharmDx assay (Agilent Technologies, Dako, Carpinteria, CA, USA). The PD-L1 tumor proportion score (TPS) was calculated as the percentage of complete or partial membrane staining in a sample included at least 100 viable cancer cells, ranging from 0 to 100% by two pathologists at our institution. Adenocarcinoma generally has tissue heterogeneity. The calculation of TPS based on heterogeneous PD-L1 expression regions was performed according to the general protocol of the 22C3 assay. The tumor area was visually divided into four regions, the percentage of PD-L1-positive cells in the four regions was measured, and the average was used as the clinical TPS. The calculated TPS was approximated as the TPS of the dominant tissue, since the dominant histological region inevitably has the most influence on the calculation of TPS. Representative images of staining of PD-L1 were shown in Supplementary Fig. S1.

Statistical analyses

We used chi-square tests to compare the proportions of categorical variables between the groups with a PD-L1 expression of <1, 1–49 and ≥50%. We used multiple regression analyses to evaluate the association between histological types and PD-L1 expression quantitatively. The number of factors included in the multivariate analysis is known to depend on the number of cases (4). A multiple regression analysis can analyze the number of total cases divided by 15, so in our study, the upper limit was 24 (356 divided by 15). The factors to be assessed were determined before the analysis was performed. In this study, there were six histological types of NSCLC: ADC, squamous cell carcinoma (SCC), adenosquamous carcinoma (ASC), large cell carcinoma (LCC), large cell neuroendocrine carcinoma (LCNEC) and pleomorphic carcinoma (PC). Furthermore, there were seven subtypes of ADC: minimally invasive ADC (MIA), lepidic ADC (Lepidic), papillary ADC (Papillary), acinar ADC (Acinar), micropapillary ADC (Micropapillary), solid ADC (Solid) and invasive mucinous ADC (IMA). The age, gender, smoking status, pathological stage (eighth edition of the tumor-node-metastasis Classification of Malignant Tumors) and *EGFR* mutation were also added as confounders in our multiple regression analysis with reference to previous studies (5,6). We considered the PD-L1 expression (i.e. TPS) as a continuous variable. When conducting the multiple regression analysis, the following points were kept in mind: the TPS for each case was log-transformed to approximate a Gaussian distribution, where 0 was replaced by 0.5, the intermediate value between 0 and the detection limit of 1. The multicollinearity between each factor was assessed by the variance inflation factor (VIF) <5 (7). The normality of the residuals of the linear regression model was confirmed by a normal quantile–quantile (Q–Q) plot. Since the partial regression coefficient (B) for each factor is a log-transformed value, the logarithm is removed by transforming B to a power of 10 and this number is presented as the result of B. Statistical analyses were conducted using Easy R (EZR) (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). EZR is a modified version of R commander with added biostatistical functions (8). All P values of <0.05 were considered statistically significant.

Results

The association between the PD-L1 expression and clinicopathological characteristics of NSCLC

The clinicopathological features classified by the PD-L1 expression are shown in Table 1. Among the clinical factors, the PD-L1 expression was higher in male ($P < 0.001$) and smokers ($P < 0.001$). Among the pathological factors, the PD-L1 expression tended to be higher in SCC, ASC and PC than in ADC ($P < 0.001$). The PD-L1 expression in pathological Stages II and III tended to be higher than in Stage I ($P = 0.003$). In cases with an *EGFR* mutation, the wild-type tended to have a higher PD-L1 expression than the *EGFR* mutations ($P < 0.001$).

The association between the histological type and PD-L1 expression in NSCLC

The association between the histological type and PD-L1 expression was evaluated by B in a multiple regression analysis adjusted for age, gender, smoking status, pathological stage and *EGFR* mutation status as confounders (Table 2). There was no multicollinearity in any of the factors (VIF < 5) (Supplementary Table S1). The normality of the residuals was assessed with a normal Q–Q plot, confirming that most of the data generally followed a 45° line (Supplementary Fig. S2).

Table 1. The association between the PD-L1 expression and clinicopathological characteristics of non-small cell lung cancer

Variables	Total (n = 356)	PD-L1 expression			P
		<1% (n = 96)	1–49% (n = 177)	≥50% (n = 83)	
Age (years), n (%)					0.140
≥70	183 (51)	50 (52)	98 (55)	35 (42)	
Gender, n (%)					< 0.001
Male	213 (59)	38 (40)	108 (61)	67 (81)	
Smoking status, n (%)					< 0.001
Current/former	235 (66)	50 (52)	111 (63)	74 (89)	
Histological type, n (%)					< 0.001
ADC	263 (74)	91 (95)	136 (77)	36 (43)	
MIA	7 (2)	7 (7)	0 (0)	0 (0)	
Lepidic	32 (9)	15 (16)	17 (10)	0 (0)	
Papillary	140 (39)	46 (48)	81 (46)	13 (16)	
Acinar	34 (10)	5 (5)	22 (12)	7 (8)	
Micropapillary	7 (2)	1 (1)	5 (3)	1 (1)	
Solid	19 (5)	1 (1)	5 (3)	13 (16)	
IMA	24 (7)	16 (17)	6 (3)	2 (2)	
SCC	62 (17)	2 (2)	28 (16)	32 (39)	
ASC	6 (2)	0 (0)	4 (2)	2 (2)	
LCC	3 (1)	0 (0)	2 (1)	1 (1)	
LCNEC PC	11 (3) 11 (3)	3 (3) 0 (0)	5 (3) 2 (1)	3 (4) 9 (11)	
Pathological stage, n (%)					0.003
I	237 (67)	74 (77)	121 (68)	42 (51)	
II	70 (20)	15 (16)	30 (17)	25 (30)	
III	49 (13)	7 (7)	26 (15)	16 (19)	
EGFR mutation, n (%)					<0.001
Positive	115 (32)	47 (49)	62 (35)	6 (7)	

PD-L1, programmed death-ligand 1; ADC, adenocarcinoma; MIA, minimally invasive adenocarcinoma; Lepidic, lepidic adenocarcinoma; Papillary, papillary adenocarcinoma; Acinar, acinar adenocarcinoma; Micropapillary, micropapillary adenocarcinoma; Solid, solid adenocarcinoma; IMA, invasive mucinous adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous carcinoma; LCC, large cell carcinoma; LCNEC, large cell neuroendocrine carcinoma; PC, pleomorphic carcinoma; *EGFR*, epidermal growth factor receptor gene.

Regarding the histological type, a significantly lower PD-L1 expression was observed among MIA (B, 0.17; 95% confidence interval: CI, 0.05–0.59), Lepidic (B, 0.46; 95% CI, 0.23–0.90) and IMA (B, 0.34; 95% CI, 0.16–0.74) patients than Papillary patients. In contrast, the PD-L1 expression was significantly higher among Acinar (B, 1.98; 95% CI, 1.05–3.76), Solid (B, 5.11; 95% CI, 2.20–11.9), SCC (B, 4.32; 95% CI, 2.45–7.62), ASC (B, 4.17; 95% CI, 1.05–16.6) and PC (B, 13.0; 95% CI, 4.43–38.2) patients than Papillary patients.

Discussion

In the present study, we evaluated the association between the PD-L1 expression and histology in NSCLC. A multiple regression analysis showed that MIA, IMA and Lepidic were weakly associated with the PD-L1 expression compared to Papillary, whereas Acinar, ASC, SCC, Solid and PC were more strongly associated than Papillary.

In the univariate analysis, gender, smoking status, pathological stage and *EGFR* mutation status were shown to be associated with the PD-L1 expression. This result shows a similar trend to previous studies (5,6) and indicates that the cohort we used for our study is not unique. Although many studies have reported an association between NSCLC histology and the PD-L1 expression, the results have remained controversial. Some studies have found that SCC has a stronger tendency to express PD-L1 than ADC (9), whereas others found no marked differences in the PD-L1 expression between SCC and ADC (10). There have been reported that the PD-L1 expression did not differ among ADC subtypes (11), whereas others found that Acinar and Solid tended to have a higher PD-L1 expression than other ADC subtypes (5). In addition, some studies reported that NSCLC with poorly differentiated histological patterns were much more likely to express PD-L1 than those with other patterns (12,13). However, these previous studies were only qualitative descriptions. Therefore, our understanding of the association between the NSCLC histology and the PD-L1 expression has remained insufficient.

In the present study, we newly showed quantitatively that the PD-L1 expression in NSCLC was dependent on the histological type using a multiple regression analysis. Regarding ADC, the PD-L1 expression in ADC subtypes increased in the order of MIA, IMA, Lepidic, Papillary, Acinar and Solid. Excluding IMA, this order is perfectly consistent with the pathologically predominant growth pattern. This result suggests that the PD-L1 expression in lung ADC subtypes depends on the degree of differentiation. Regarding IMA, a previous study has reported that IMA tended to have a low PD-L1 expression (14). However, our multiple regression analysis newly showed that IMA was likely to have a lower expression of PD-L1 than well-differentiated invasive ADC, such as Lepidic. Since IMA has been reported to express an mRNA that encodes a protein product thought to regulate a different immune checkpoint than PD-L1 (15), immune checkpoint inhibitors targeting different proteins other than PD-1/PD-L1 may be necessary for treating IMA.

Table 2. Histological type dependence of the PD-L1 expression in non-small cell lung cancer according to a multiple regression analysis

Histological type	B [a] (95% CI)	*P*	VIF
ADC			
Papillary	Reference	—	—
MIA	0.17 (0.05–0.59)	0.01	1.24
Lepidic	0.46 (0.23–0.90)	0.02	1.12
Acinar	1.98 (1.05–3.76)	0.04	1.18
Micropapillary	1.03 (0.29–3.67)	0.97	1.05
Solid	5.11 (2.20–11.9)	< 0.001	1.19
IMA	0.34 (0.16–0.74)	0.01	1.25
SCC	4.32 (2.45–7.62)	< 0.001	1.54
ASC	4.17 (1.05–16.6)	0.04	1.06
LCC	5.67 (0.81–39.3)	0.08	1.05
LCNEC	0.85 (0.29–2.44)	0.76	1.13
PC	13.0 (4.43–38.2)	< 0.001	1.16

Adjusted R^2 0.30, *P* value <0.001.

[a]Since the PD-L1 expression was log-transformed in the multiple regression analysis, the partial regression coefficient (B) is shown to the power of 10.

PD-L1, programmed death-ligand 1; B, partial regression coefficient; CI, confidence interval; VIF, variance inflation factor; ADC, adenocarcinoma; Papillary, papillary adenocarcinoma; MIA, minimally invasive adenocarcinoma; Lepidic, lepidic adenocarcinoma; Acinar, acinar adenocarcinoma; Micropapillary, micropapillary adenocarcinoma; Solid, solid adenocarcinoma; IMA, invasive mucinous adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous carcinoma; LCC, large cell carcinoma; LCNEC, large cell neuroendocrine carcinoma; PC, pleomorphic carcinoma.

Regarding the histology other than ADC, ASC, SCC and PC tended to have higher PD-L1 expression in that order. Since SCC have been reported to possess a moderate to poorly differentiated histological pattern (16), the expression of PD-L1 in NSCLC other than ADC may reflect a poorly differentiated histology. Regarding LCC and LCNEC, which have been reported to have a low PD-L1 expression (5,17), our analysis did not show any significant differences between the expression of PD-L1 and their histological types. To our knowledge, this study is the first to analyze the PD-L1 expression in NSCLC of different histological types and to illustrate the differences quantitatively with numeric values by a multiple regression analysis.

The association suggested by our study may be biologically explicable. It has been reported that *TP53* mutations in NSCLC are associated with the expression of PD-L1 (18). As a molecular mechanism, it has been reported that p53 in NSCLC downregulates the expression of PD-L1 via miR-34a, which is a MicroRNA that is encoded by the *MIR34A* in humans (19). It has also been reported that the frequency of *TP53* mutations in lung adenocarcinoma tends to be higher in poorly differentiated histological types (20). Therefore, the tissue differentiation dependence of PD-L1 expression in NSCLC revealed in our simple mathematical model may be related to *TP53* mutation for molecular mechanism.

Several limitations associated with the present study warrant mention. First, this study was a single-center retrospective analysis with a small sample size. The number of cases by individual histological types needs to be further collected and analyzed. Second, our study was limited to surgical cases; we did not examine advanced cases without surgical application. The PD-L1 expression in Stage IV may behave differently than in other stages. A more detailed understanding of the histological dependence of the PD-L1 expression may also require an analysis of Stage IV cases. Third, the molecular mechanism underlying the histological type dependence of the PD-L1 expression in NSCLC, as revealed by our statistical approach, is unclear. Based on the findings of previous studies, the *TP53* mutation may be involved, but further studies will be needed in order to confirm this.

In conclusion, we quantitatively analyzed the PD-L1 expression among NSCLCs using a mathematical model of a multiple regression analysis. We confirmed that the expression of PD-L1 in NSCLC differed greatly depending on the histological type. This trend in the PD-L1 expression in NSCLC may be useful for screening patients when considering the application of immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway.

Abbreviations

ADC, adenocarcinoma; Acinar, acinar adenocarcinoma; ASC, adenosquamous carcinoma; *EGFR*, epidermal growth factor receptor gene; IMA, invasive mucinous adenocarcinoma; LCC, large cell carcinoma; LCNEC, large cell neuroendocrine carcinoma; Lepidic, lepidic adenocarcinoma; MIA, minimally invasive adenocarcinoma; Micropapillary, micropapillary adenocarcinoma; PC, pleomorphic carcinoma; PD-L1, programmed death-ligand 1; Papillary, papillary adenocarcinoma; SCC, squamous cell carcinoma; Solid, solid adenocarcinoma

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Conflict of interest statement

Kensuke Kojima has nothing to disclose.
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